

TECHNICAL NOTES

Rarefaction of Benthic Macroinvertebrate Taxonomic Lists:

Impact of Changes to the Taxon List and on B-IBI Scores



SCOTT CREEK BENTHIC SAMPLING



WESTERN PEARLSHELL MUSSEL IN SCOTT CREEK

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*Sampling Reach and a Western Pearlshell Mussel:
Snohomish County Surface Water Management*

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Executive Summary

The Snohomish County Surface Water Management (SWM) team implementing the State of Our Waters Program (SOW) reports on the condition of at least thirty streams per year. These randomly selected sites include ten or more new sites each year and re-visits to twenty long-term, trend monitoring sites. Benthic macroinvertebrates are collected at each of these sites and analyzed using B-IBI scores as an indicator for determining site biological condition.

As an attempt to standardize site assessment expressions using the B-IBI score, the Puget Sound Stream Benthos Database (PSSB) uses a rarefaction routine that recalculates biometric scores based on a 500-count benthic sample versus the actual sample count reported by the taxonomic laboratory (usually a count between 500 and 600 organisms). Several organizations submit sample data to the PSSB database, with some using different collection devices, but all attempt to maintain the same area sampled per visited stream reach (e.g., 8-square feet).

The objective of this analysis was to determine :

1. B-IBI scoring differences for five test sites using the Puget Sound Stream Benthos (PSSB) database output versus biometrics generated by our taxonomic consultant using all of the sub-sampled species/densities;
2. Which of the biometrics components of the B-IBI score have different values between methods of calculation or are influenced by rarefaction; and
3. How biological condition, as expressed through B-IBI, differs between rarefied samples (reduction to 500-count) versus full-count sample analysis.

Generation of B-IBI scores using either full-count data or rarefaction showed no difference in condition category determination (Very Poor, Poor, Fair, Good, Excellent). However, there were some large difference between B-IBI scores at Excellent sites even when both methods resulted in determining a site in Excellent condition.

Comparison of B-IBI rarefied samples with full-count samples showed a difference between biometrics that are richness-based. Richness-based biometrics are counts of species in a sample that belong to a taxonomic group (e.g., Ephemeroptera) and other group designations that display a specific ecological characteristic, like Long-Lived. The ecological characteristic where differences were found between rarefied and full-count samples varied by condition category.

Sites with B-IBI scores that correspond to Excellent condition had lower Intolerant Taxa Richness and Long-Lived Taxa Richness and had consistently lower B-IBI scores with full-count data sets. This was problematic in that sites with rarefied data had the same or lower Total Taxa Richness compared to full-count data sets, yet more taxa in select richness biometrics were found. This comparison of total taxa richness and identity of taxa between rarefied and full-count data sets meant that designation of Intolerant Taxa and Long-Lived Taxa was different in the PSSB database from our taxonomic consultants designations.

Sites with B-IBI scores that correspond to Fair condition category had consistently higher B-IBI scores with full-count data. Biometrics responsible for these differences were Percent Predator and Percent Dominant. Biometric scores for both expressions were consistently higher for the full-count data over those following rarefaction. These biometrics were more influential in determining B-IBI scores with full

count samples at sites where individual taxa abundance could be very high. When rarefaction is used, these abundant taxa are largely ignored and do not contribute as much to a higher B-IBI score.

The SOW Program relies on long-term consistency and assessment tools that have regional application. Taxonomy, function, and behavior of each species in the aquatic environment need to be consistently recognizable among monitoring organizations before spatial patterns or trends can be identified using an aggregate of results. Given the scientific knowledge base for taxon autecology (behavior and function) explains distribution and abundance in the environment, changes of these designations through continuing research is inevitable and we need to be aware of this impact on linking causal factors with change over space and time.

To overcome this problem, we recommend taxa designations remain consistent for trend analysis or be updated on a ten-year basis (or less, when warranted) to include advancements in knowledge of taxa autecology. Changes to what we know of benthic macroinvertebrate taxa roles in stream ecosystems should be carefully annotated now so that future data analysis is informed on how to revise data generated in the past for comparison with current data. Rarefaction is ideally used when species identity and number of taxa are compared between sites. Because we use a multi-metric index for assessment that contains additional expressions not related to number of taxa, reduction of a taxa list or density estimates of individual taxa has an effect on the assessment score. If we consistently processed whole samples and had large differences in density estimates, rarefaction could be used to equalize for the paucity of individuals.

1.1 Background

1.1 What is Rarefaction

Rarefaction is a widely applied technique for comparing the species richness (number and density¹ of taxa) of scientific samples that differ in area, volume or sampling effort. It is a statistical re-sampling technique that standardizes an ecological community dataset to a predetermined standard count. The reason rarefaction is used to describe community data is that it allows the comparison of samples that have unequal sub-sample or counts. For example, one sub-sample may count 500 individuals while another sub-sample may count 700 individuals. The assumption that occurs when sampling various species in a community is that the larger the number of individuals counted, the more species that will be found, although this is not always the case. Rarefaction of a sub-sample is assumed to eliminate this bias. Rarefaction can result in exclusion of some taxa and a reduction of estimated densities for individual taxa.

1.2 When is Rarefaction Used

Rarefaction can be used to produce comparable biological information when either: 1) samples with unequal collection areas are compared, or 2) the total number of individuals counted from a sample are different. Comparison of samples that have drastically different collection areas (e.g., 0.05-0.1 m², 0.1-0.5m², 0.5-1.0m², or >1.0m²) will produce different numbers of taxa when sub-sampled (Vinson and Hawkins 1996). This is especially true when sample collection area and/or sub-sampling count is small for a site.

Standardizing datasets based on the number of organisms produces different results than standardizing based on sampling area (Gotelli and Colwell 2001). When datasets are standardized based on sampling area, the resulting taxa list may be biased toward fewer species. This is because individuals are not uniformly distributed throughout a stream reach and therefore, not represented in all samples.

Alternatively, standardizing datasets based on the number of individuals, or fixed-count, begins with a larger species list and is not effected by distribution patterns.

Sample area rarefaction can produce different results than fixed-count rarefaction when estimating species richness. The greatest influence that explains this difference is when sample area comparisons are unequal, and representation is from a variety of habitats in the stream reach. Another factor that effects fixed-count rarefaction is disturbance. Disturbed communities can have evenly distributed taxa (same tolerant taxa everywhere) and a reduction of taxa density (with chronic toxicity). Rarefaction effectively over-represents proportion of rare species, when present, in disturbed site samples because of this uniform distribution. This can lead to disturbed communities condition being biased high because a fundamental assumption for richness metrics is that greater taxa richness reflects higher quality conditions. The unintended effect of using fixed-count rarefaction at degraded sites may diminish an identifiable biotic signature warning of an impaired benthic community (McCabe and Gotelli 2000).¹

¹ Enumeration of individual taxa is known as “relative abundance” and is based on a sub-sample of the total collected. The term “density” reflects enumeration of a whole sample from the area benthos have been collected. Density has been used in place of relative abundance throughout this document as it is a term more recognizable by a broader audience.

1.3 Rarefaction Applied to Puget Sound Stream Benthos Data

One of the primary tools used to calculate B-IBI scores for Puget Sound streams is the Puget Sound Stream Benthos (PSSB) database (King County 2009). The PSSB database generates individual metric scores and an overall B-IBI score from rarefied data. There is an option to generate metric scores and the overall B-IBI score using the whole sub-sampled data.

A primary assumption for use of rarefaction with Puget Sound benthic samples (Puget Sound Stream Benthos database) is that B-IBI scores are higher when more individuals from the sample are counted. Standardizing the bug count to 500 organisms ensures that each sample is handled equitably and that comparisons can be made between any of the site assessments stored in the database. This may not be true in cases where some species have a greater number of individuals present in the sample. Seven of the biometrics in the Puget Sound Benthic Index of Biological Integrity (B-IBI) are species richness expressions (structural attributes). If rarefaction of a sample reduces the number of species in order to achieve a threshold (500-count), richness metrics can be effected. When density of individual species is changed through rarefaction the three percentage-based metrics can be effected.

There are many permutations of rarefaction that include consideration of: 1) fixed count sub-sampling, 2) weighting of select taxa, and 3) an attempt to retain unique taxa. Rules used for a rarefaction equation vary, but a basic routine is presented in Appendix 1 and is not identical to that used by the Puget Sound Benthos Database (PSSB).

1.4 Goals of this Review and Analysis

This analysis examines the impact of rarefaction on samples when using a standard bug count of 500 individuals and how exclusion of taxa and/or the reduction of densities changes the estimates of individual biometrics and index scores. Results for individual metrics and B-IBI scores with a rarefied dataset and others provides some insight into the influence individual taxa have on biometric scores and the B-IBI.

The objective of the following analysis is to determine:

1. B-IBI scoring differences for five test sites using the Puget Sound Stream Benthos (PSSB) database output versus biometrics generated by our taxonomic consultant using all of the sub-sampled species/densities;
2. Which of the biometrics components of the B-IBI score have different values between methods of calculation and are influenced by rarefaction; and
3. How biological condition differs between rarefied samples (reduction to 500-count) versus whole sub-sample analysis.

2 Approach

This section provides detailed information on how benthic macroinvertebrates are sampled in the field, taxa are identified in the laboratory, and data are analyzed for generation of biometrics and B-IBI score. This is important background information that can explain why differences may occur when using rarefied datasets and if elements of monitoring and identification are factors that explain these differences. Additionally, this background information will be the basis for evaluating information loss

from rarefaction and if this loss is ecologically relevant. Because B-IBI scores are used to make regulatory decisions, the methods used for calculating scores needs to be fully evaluated from preparation of the data to calculation of biometrics.

2.1 Sample Collection Protocols

Collection of benthic macroinvertebrate samples targets riffle habitat in the Snohomish County State of Our Waters (SOW) Monitoring Program (SWM 2019). Larsen (2019) lists several strategies for characterizing benthic conditions in a stream reach and identified the targeted riffle collection using a Surber sampler appropriate for regulatory decision-making. Riffle habitat usually hosts more species than other habitat types (e.g., pools and glides). Assessment tools can reliably detect subtle changes when more taxonomic information (i.e., more species) is used to determine health of the community. There are several other reasons why Snohomish County targets riffles and uses the Surber sampler for collection of benthic macroinvertebrates:

- Sampling with a Surber requires careful handling and collection of benthic macroinvertebrates from substrate particles;
- Isolating collection within a single habitat-type, riffles, can identify specific types of impacts from swift running water habitat;
- There are usually more taxa in riffle habitat which improves the sensitivity allowing for the detection of minor changes in water quality or habitat conditions when a pollutant is present;
- Repeatability of results with replicate samples in riffles is greater and reduces variability in the collection of benthic samples among field staff; and
- Assessment tools have been developed and calibrated for swift running water (riffle and glides) habitat.

Comparison of data sets for trend analysis or identifying patterns of effectiveness in stream restoration projects requires consideration of habitat type sampled (or composited), area of benthos sampled, and level of taxonomic effort used to generate taxonomic results (see section 2.4 for more detail).

2.2 Laboratory Analysis Methods

2.2.1 Benthic Macroinvertebrates

Taxonomic effort for identifying benthic macroinvertebrates in samples collected beginning in 1997 may differ from the taxonomic effort used for identification of species in the 2019 samples. The initial surveys completed in 1997-2001 used the following guidelines for taxonomic effort:

- Individuals identified to species, where possible;
- Chironomids were identified to Genus; and
- Other taxonomic groups were identified to Class or Order.

Twenty years later, taxonomic revisions have occurred recognize species that were previously only identified to Genus and additional knowledge about function and behaviour of individual taxa in the aquatic environment.

A detailed list of current taxa designations that are the basis for calculating individual biometrics and a B-IBI score in the PSSB database can be found at: <https://www.pugetsoundstreambenthos.org/Taxa-Attributes.aspx>.

2.3 Data Analyses

2.3.1 Benthic Macroinvertebrates

The Benthic Index of Biological Integrity (B-IBI) was used to generate a score for each of the samples collected from a site. B-IBI scores were calculated once biometric scores were converted to a 0-10 score and summed for a total possible high score of 100 units.

B-IBI scores are based on ten biometrics that reflect the response from a component of the benthic macroinvertebrate community (Table 1). Table 1 also explains the utility of each biometric and the expected direction of change when factors that degrade physical habitat or water quality is present.

TABLE 1. BIOMETRICS INCLUDED IN THE PUGET SOUND BENTHIC INDEX OF BIOTIC INTEGRITY (B-IBI).

Biometric	Definition	Predicted Response to Impairment
Total Taxa Richness (Count of all taxa)	A measure of the number of kinds of organisms (taxa) in a collection.	Decrease in number of taxa.
Ephemeroptera Taxa Richness (Count of Ephemeroptera taxa)	Number of mayfly taxa.	Decrease in number of mayfly taxa as mayflies are pollution-sensitive, with the exception of the Baetidae family.
Plecoptera Taxa Richness (Count of Plecoptera taxa)	Number of stonefly taxa.	Decrease in number of taxa as stoneflies are pollution-sensitive.
Trichoptera Taxa Richness (Count of Trichoptera taxa)	Number of caddisfly taxa.	Decrease in number of taxa as caddisflies are pollution-sensitive, with the exception of the Limnephilidae family.
Intolerant Taxa Richness (Count of intolerant taxa)	Number of genera that are sensitive to pollutants.	Decrease in number of pollution-sensitive genera.
Clinger Taxa Richness and Percent (Count of Clinger taxa and percent of the total sample)	Number of taxa that cling to smooth surfaces in fast water, and live in spaces between rocks in the stream bed.	Decrease in number of taxa due to disturbance of streambed sediment or deposition of fine sediment.
Long-Lived Taxa Richness (Count of long-lived taxa)	Number of taxa that live multiple years in the water before leaving the water as adults.	Decrease in number of taxa that live two or more years in the water.
Percent Tolerant	Percentage of taxa that are more tolerant of pollution.	Usually an increase in percentage of pollution-tolerant taxa.
Percent Predator	Percentage of taxa that prey on other aquatic organisms.	Decrease in percentage of predatory taxa.
Percent Dominance	Percentage of the single most abundant taxon relative to other taxa.	Higher percentage of the most abundant taxon, which with impairments to a stream tends to be an impairment-tolerant taxon.

Source: Plotnikoff and Blizard (2013)

Five biological condition scoring ranges are identified in Table 2. The scores are determined by adding all ten biometric scores, on a scale of 0-10, calculated from the benthic macroinvertebrate dataset. Table 2 describes the scoring range condition in greater detail by including changes that occur for benthic macroinvertebrate indicators. These descriptions are generalizations and additional changes to indicators may be identifiable at any one of the sites assessed using the B-IBI score.

TABLE 2. B-IBI SCORE INFORMATION.

Score range classification	Score range	General description of aquatic life in score range
Excellent	80 – 100	<ul style="list-style-type: none"> • Comparable to least disturbed reference condition • Overall high taxa diversity, particularly of mayflies, stoneflies, caddisflies, and long-lived, clinger, and intolerant taxa • High relative abundance of predators
Good	60 – 79	<ul style="list-style-type: none"> • Slightly divergent from least disturbed condition • Absence of some long-lived and intolerant taxa • Slight decline in richness of mayflies, stoneflies, and caddisflies • Increased proportion of tolerant taxa
Fair	40 – 59	<ul style="list-style-type: none"> • Total taxa richness reduced, particularly intolerant, long-lived, stonefly, and clinger taxa • Reduced relative abundance of predators • Proportion of tolerant taxa continues to increase
Poor	20 – 39	<ul style="list-style-type: none"> • Overall taxa diversity reduced • Greatly reduced proportion of predators and long-lived taxa richness • Few stoneflies or intolerant taxa present • Dominance by three most abundant taxa often very high
Very Poor	0 – 19	<ul style="list-style-type: none"> • Overall taxa diversity very low and dominated by a few highly tolerant taxa • Mayfly, stonefly, caddisfly, clinger, long-lived, and intolerant taxa largely absent • Relative abundance of predators very low

Information source: Puget Sound Stream Benthos website <https://www.pugetsoundstreambenthos.org/About-BIBI.aspx>

2.3.2 Analytical Approach: Reason(s) for Differing B-IBI Scores

Five sites where benthic macroinvertebrates were collected by SWM in 2018 and submitted to the Puget Sound Stream Benthos (PSSB) database were used for this analysis. Output of the biometric calculations and the B-IBI score were compared with biometrics reported from Rhithron Associates, Inc., Snohomish County's taxonomic consulting laboratory. The original data submitted to the PSSB database was analyzed for the identification of taxa with density estimates for each.

If exclusion of taxa from rarefaction of a sub-sample, individual biometrics could be effected and, ultimately, the B-IBI score. For some biometrics, these differences might effect simple counts like: Total Taxa Richness, Plecoptera Richness, or Ephemeroptera Richness, Trichoptera Richness, Long-lived Taxa Richness, Intolerant Richness, and Clinger Richness (see Table 1). Remaining biometrics in the list of ten

that are influenced by changes to density estimates of taxa in the sub-sample include Percent Tolerant, Percent Predator, and Percent Dominance.

A feature of the PSSB database is that rarefaction is used to re-sample sites where the taxonomic lab counted more than 500 organisms. When rarefaction is used to generate a new set of taxa and/or abundances, some taxa may be eliminated from further consideration and that can alter the biometric scores and, ultimately, the B-IBI score. The more common taxa are likely to be counted more frequently leaving rare taxa to be counted last or not at all in the case rarefaction is based on sample-count. Rarefaction of a dataset can result in a dramatic effect on the B-IBI score, either higher or lower.

In the case of the PSSB database, rarefaction is not a basic algorithm, rather, incorporates additional data handling features. These include the following: 1) sub-sampling count (e.g., 500 organisms), 2) weighting of select taxa, and 3) an attempt to retain unique taxa. Some attributes assigned by PSSB to taxa (e.g., Long-Lived or Tolerant Taxa) may differ from those used by other B-IBI calculators and could be another reason for differences in addition to effects of rarefaction.

The following analyses attempt to determine why differences occur in biometric calculations and B-IBI scores when data are rarefied versus used without this transformation.

3 Results

3.1 B-IBI Score Comparisons

There were B-IBI score differences between the PSSB database and the manual calculation equations for each of the sites (Table 3). The smallest differences were found at sites with highest and lowest B-IBI scores (e.g., FCSP and TRFT). Despite differences between each of the couplet B-IBI scores, the biological condition assessment was the same for each (e.g., FCSP = Excellent, SCTT = Excellent, WDOP = Excellent, NCCW = Fair, TRFT = Fair) (Figure 1).

TABLE 3 COMPARISON OF B-IBI SCORES FROM 2018 STATE OF OUR WATERS SAMPLING AS REPORTED IN THE PSSB DATABASE (RAREFIED) AND CALCULATED MANUALLY (NOT RAREFIED) BY USING THE REGRESSION EQUATIONS FOR CONVERSION FROM THE 10-50 SCALE TO THE 0-100 SCALE.

SITE NAME	Total Bug Count (no.'s)	Not Rarefied B-IBI	Rarefied B-IBI	DIFFERENCE	ABSOLUTE DIFFERENCE
FCSP	563	94	97	3	3
SCTT	556	82	94	12	12
WDOP	529	83	89	6	6
NCCW	552	59	49	-10	10
TRFT	315	43	42	-1	1

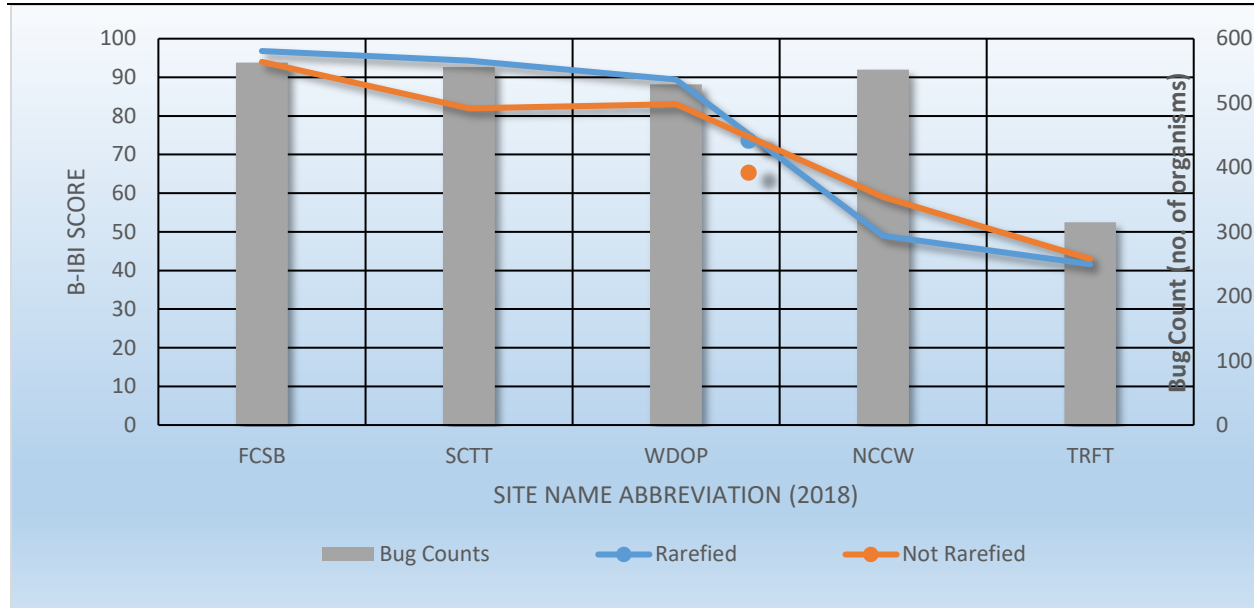


FIGURE 1. COMPARISON BETWEEN B-IBI SCORES BASED ON RAREFIED, NOT RAREFIED, AND TOTAL BUG COUNTS FROM SUB-SAMPLING.

We should note the biometric score for each site, despite any differences based on source of calculation, remained in the same biological condition category (e.g., Fair or Excellent). Since rarefaction is used in the PSSB database prior to calculating biometrics and then the B-IBI score, a comparison of the bug count beyond the 500-count minimum picked from whole samples in the laboratory was included in Figure 1. Rarefaction analysis that results in elimination of a larger number of individual organisms may result in greater differences from a sample that used all organisms beyond the 500-count. Alternatively, another postulation is that sample counts below the 500-count, and not rejected, are not altered and the biometric scores as well as the B-IBI score are identical. Results of these comparisons for five sites are reported in Table 4 through Table 8.

TABLE 4 COMPARISON OF BIOMETRIC SCORES (0-10) FOR NCCW (2018) REPORTED BY PSSB VERSUS THOSE DETERMINED BY RHITHRON TAXONOMIC CONSULTING AS REPORTED IN THE SITE TAXONOMIC REPORT.

BIOMETRIC NAME	OBSERVED VALUE_SNOCO Full Count	OBSERVED VALUE_PSSB Rarefied	OBSERVED VALUE_PSSB Full Count	SNOCO_B-IBI SCORE (0-10)	PSSB_B-IBI SCORE (0-10)
Total Taxa Richness	33	32	32	2	2
Ephemeroptera Richness	4	4	4	4	4
Plecoptera Richness	5	5	5	6	6
Trichoptera Richness	7	6	6	8	6
Long-lived Richness	5	6	6	4	5
Intolerant Richness	1	1	1	1	1

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BIOMETRIC NAME	OBSERVED VALUE_SNOCO Full Count	OBSERVED VALUE_PSSB Rarefied	OBSERVED VALUE_PSSB Full Count	SNOCO_B-IBI SCORE (0-10)	PSSB_B-IBI SCORE (0-10)
Percent Tolerant	11.78	14	12.7	7	7
Percent Predator	18.12	11.4	11.8	9	5
Clinger Richness	21	20	20	8	8
Percent Dominant	26.99	50	45.7	10	5

TABLE 5 COMPARISON OF BIOMETRIC SCORES (0-10) FOR SCTT (2018) REPORTED BY PSSB VERSUS THOSE DETERMINED BY RHITHRON TAXONOMIC CONSULTING AS REPORTED IN THE SITE TAXONOMIC REPORT.

BIOMETRIC NAME	OBSERVED VALUE_SNOCO Full Count	OBSERVED VALUE_PSSB Rarefied	OBSERVED VALUE_PSSB Full Count	SNOCO_B-IBI SCORE (0-10)	PSSB_B-IBI SCORE (0-10)
Total Taxa Richness	58	55	55	10	10
Ephemeroptera Richness	8	8	8	10	10
Plecoptera Richness	6	6	6	7	7
Trichoptera Richness	12	11	11	10	10
Long-lived Richness	6	10	10	5	10
Intolerant Richness	2	6	6	3	9
Percent Tolerant	12.41	2.4	2.3	7	9
Percent Predator	33.45	35.6	33.1	10	10
Clinger Richness	29	26	26	10	10
Percent Dominant	15.11	33.8	31.1	10	9

TABLE 6 COMPARISON OF BIOMETRIC SCORES (0-10) FOR WDOP (2018) REPORTED BY PSSB VERSUS THOSE DETERMINED BY RHITHRON TAXONOMIC CONSULTING AS REPORTED IN THE SITE TAXONOMIC REPORT.

BIOMETRIC NAME	OBSERVED VALUE_SNOCO Full Count	OBSERVED VALUE_PSSB Rarefied	OBSERVED VALUE_PSSB Full Count	SNOCO_B-IBI SCORE (0-10)	PSSB_B-IBI SCORE (0-10)
Total Taxa Richness	63	62	62	10	10
Ephemeroptera Richness	10	10	10	10	10
Plecoptera Richness	8	7	7	10	9
Trichoptera Richness	9	9	9	10	10
Long-lived Richness	8	9	9	8	9
Intolerant Richness	1	4	4	1	6
Percent Tolerant	15.69	2.0	1.9	6	9

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BIOMETRIC NAME	OBSERVED VALUE_SNOCO Full Count	OBSERVED VALUE_PSSB Rarefied	OBSERVED VALUE_PSSB Full Count	SNOCO_B-IBI SCORE (0-10)	PSSB_B-IBI SCORE (0-10)
Percent Predator	16.45	14.6	14	8	7
Clinger Richness	33	33	33	10	10
Percent Dominant	10.96	28.0	28.9	10	10

TABLE 7 COMPARISON OF BIOMETRIC SCORES (0-10) FOR TRFT (2018) REPORTED BY PSSB VERSUS THOSE DETERMINED BY RHITHRON TAXONOMIC CONSULTING AS REPORTED IN THE SITE TAXONOMIC REPORT.

BIOMETRIC NAME	OBSERVED VALUE_SNOCO Full Count	OBSERVED VALUE_PSSB Rarefied	OBSERVED VALUE_PSSB Full Count	SNOCO_B-IBI SCORE (0-10)	PSSB_B-IBI SCORE (0-10)
Total Taxa Richness	32	32	32	2	2
Ephemeroptera Richness	3	3	3	3	3
Plecoptera Richness	3	3	3	3	3
Trichoptera Richness	4	4	4	4	4
Long-lived Richness	8	7	7	8	6
Intolerant Richness	0	2	2	0	3
Percent Tolerant	39.37	1.3	1.3	1	10
Percent Predator	24.13	13.7	13.7	10	6
Clinger Richness	14	16	16	4	5
Percent Dominant	38.1	74.6	74.6	8	0

TABLE 8 COMPARISON OF BIOMETRIC SCORES (0-10) FOR FCSP (2018) REPORTED BY PSSB VERSUS THOSE DETERMINED BY RHITHRON TAXONOMIC CONSULTING AS REPORTED IN THE SITE TAXONOMIC REPORT.

BIOMETRIC NAME	OBSERVED VALUE_SNOCO Full Count	OBSERVED VALUE_PSSB Rarefied	OBSERVED VALUE_PSSB Full Count	SNOCO_B-IBI SCORE (0-10)	PSSB_B-IBI SCORE (0-10)
Total Taxa Richness	58	58	58	10	10
Ephemeroptera Richness	8	8	8	10	10
Plecoptera Richness	12	12	12	10	10
Trichoptera Richness	10	10	10	10	10
Long-lived Richness	9	12	12	9	10
Intolerant Richness	4	7	8	6	10

BIOMETRIC NAME	OBSERVED VALUE_SNOCO Full Count	OBSERVED VALUE_PSSB Rarefied	OBSERVED VALUE_PSSB Full Count	SNOCO_B-IBI SCORE (0-10)	PSSB_B-IBI SCORE (0-10)
Percent Tolerant	6.22	1.6	1.4	9	10
Percent Predator	20.43	17	17.2	10	8
Clinger Richness	34	30	30	10	10
Percent Dominant	12.08	35	31.1	10	9

A summary of differences between the SNOCO and PSSB biometrics are reported in Table 7. In cases where a negative value appears, the biometric score used by SNOCO (full-count) was lower than reported by PSSB rarefied analysis. In cases where a positive value appears, the biometric score used by SNOCO was higher than reported by PSSB . Addition of these differences (negative and positive values) represented the total difference between the two methods used to calculate B-IBI scores (Table 9).

TABLE 9 BIOMETRIC SCORES THAT DIFFERED BETWEEN PSSB (RAREFIED) AND SNOCO (FULL COUNT).

Site/Metric	Total Bug Count (no.'s)	Total Taxa Richness	Ephemeroptera Richness	Plecoptera Richness	Trichoptera Richness	Long-Lived Richness	Intolerant Richness	Percent Tolerant	Percent Predator	Clinger Richness	Percent Dominant	Total Difference
NCCW	552				X (+2)	X (-1)			X (+4)		X (+5)	+10
SCTT	556					X (-5)	X (-6)	X (-2)			X (+1)	-12
WDOP	529			X (+1)		X (-1)	X (-5)	X (-3)	X (+1)			-7
TRFT	315					X (+2)	X (-3)	X (-9)	X (+4)	X (-1)	X (+8)	+1
FCSP	563					X (-1)	X (-4)	X (-1)	X (+2)		X (+1)	-3

Note: The number and +/- in parentheses indicates the difference and direction, higher (+) or lower (-), of the SNOCO result from the PSSB result (e.g., +1 means the SNOCO biometric score was 1 unit higher than the PSSB biometric score).

3.2 Taxonomic Differences between Sites

Taxonomic lists for each site are in Appendix 2 through Appendix 6. Taxa excluded by rule for calculation of the B-IBI score included adults and pupa. Very few taxa were excluded by PSSB rarefaction routine. Rather, changes occurred mostly in reduction of density estimates for individual taxa in order to meet the 500-count standardization goal.

The biometrics having the greatest influence on the B-IBI score were percentage expressions like: 1) Percent Tolerant Taxa, and 2) Percent Dominant Taxon (Table 9). These expressions showed greatest numerical differences between the PSSB and Rhithron Associates, Inc. biometrics . Differences in taxon identity or abundance would easily influence the output of these biometrics when they are eliminated during the rarefaction process. The biometric scores resulting from the removal of the numbers of tolerant organisms were markedly different between the PSSB rarefied sample and the whole sample used for calculation of the B-IBI score by SNOCO.

Scott Creek (5) had a greater number of reported taxa using the full-count dataset than did the rarified dataset (PSSB). In another case, French Creek at Spada Rd. had the same number of reported taxa for both the full-count and rarified dataset. In both cases, there was a greater number of Long-Lived Taxa and Intolerant Taxa reported from the rarified dataset. This discrepancy was the same for remaining sites, but not as great a difference in these richness biometrics.

3.2.1 Effects of Sample Rarefaction on Biological Information in a Sample

Use of rarefaction of samples in the PSSB Database has its purpose embedded in making all submitted samples comparable based on number of individuals in a sample (fixed-count of 500 organisms). Comparison of samples or aggregation of samples from this database is possible if they are expressed on the same basis; the fixed 500-count sample in the case of the PSSB database.

Obvious impacts rarefaction can have on taxonomic information and associated characteristics of the sampled community are:

1. Exclusion of some taxa;
2. Changes in taxon density; and
3. Alteration of functional and/or behavioral information with remaining taxa.

Comparison of whole sample and rarefied sample B-IBI scores did not differ with respect to membership to a biological condition category. This is an important result in that site assessment was not altered due to differences in how data was prepared before calculation of the B-IBI score. However, differences between B-IBI scores due to recognition (or lack of) Long-Lived species or Intolerant species are notable between PSSB database and data used by SNOCO to manually calculate a B-IBI score. Biometrics based on percentage of organisms in the community like: Percent Tolerant, Percent Dominant, and Percent Predator were effected, to a lesser extent, by taxa exclusion and mostly by the reduction of individual taxa density following rarefaction.

4 Discussion and Conclusions

4.1 Rarefied versus Full Count Sample Results

In cases where sites with B-IBI scores were in Excellent biological condition (B-IBI = 80-100), rarefaction resulted in lower or no difference in total taxa richness yet richness increased in at least two of the biometrics, Intolerant Richness and Long-Lived Richness. This difference is due to how the two sources for taxonomic information (e.g., PSSB and SNOCO) designate individual taxa that are intolerant to pollution and are long-lived (two or more years in the aquatic phase).

For sites that scored in Excellent condition, richness biometrics like Intolerant Taxa and Long-lived Taxa showed higher scores when Total Taxa Richness decreased in the rarefied PSSB dataset. Since rarefaction can result in either of reduced species in the dataset or reduced density of individual taxa or both, the rarefied PSSB dataset should not show an increased number of taxa at either of these sites. The additional taxa reported in these two biometrics indicates that PSSB recognizes more taxa with these characteristics. The consequence of these differences means that results are not comparable. Combining data from both sources for long-term trend analysis would require standardization of a recognized taxonomic list, functional and behavioral designations.

For sites that scored in Fair condition, rarefied samples had lower B-IBI scores, so the opposite case as sites in Excellent condition. This was partially due to the effects of the reduction in the density of certain taxa (NCCW and TRFT). Percent Predator decreased and Percent Dominant biometrics increased consistently at NCCW and TRFT in the rarefied PSSB results.

4.2 Guide for Use of Rarefaction

When rarefaction of a sample using a fixed-count is used as the standardized endpoint, impact on individual biometrics should be examined by comparing against biometrics from full-count data. Otherwise, the results from the rarefied dataset may result in an erroneous assessment of site condition and influence conclusions about:

- Biological condition of a site;
- Large differences in B-IBI scores at a site between years; and
- Patterns or trends in biological condition.

Rarefaction was shown to impact richness biometrics resulting from recognition of fewer species and this can result in lower B-IBI scores for high quality sites. (Figure 1). When a dataset consistently underpredicts the number of species at high quality sites, managers may set expectations for degraded site improvements much lower than would otherwise be expected.

Dominance and percentage-based biometrics were also shown to differ following rarefaction. One reason was due to a lack of consistency between organizations (Taxonomic Consultants, Databases, Numeric Models) in the categorization of taxa by structural, functional, and behavioral designations. For example, *Sweltsa* sp. (a stonefly) was categorized as Long-Lived in the PSSB database, but is not recognized as a

Long-Lived taxon in the SNOCO dataset (Table 10). The example in Table 10 shows how the number of Long-Lived and Intolerant Taxa designations differ between these taxonomic lists (SNOCO versus PSSB). Metric values for Long-Lived Taxa and Intolerant Taxa correspond with values reported in Table 5. These differences are a major reason why biometric scores do not agree and overall B-IBI scores may differ.

TABLE 10 COMPARISON OF LONG-LIVED TAXA AND INTOLERANT TAXA FROM SCOTT CREEK (SCTT 2018) USED TO CALCULATE B-IBI SCORES (SNOCO FULL-COUNT TAXA LIST AND PSSB RAREFIED TAXA LIST).

Taxon	Long-Lived Taxa		Intolerant Taxa	
	Full-Count_SNOCO	Rarefied_PSSB	Full-Count_SNOCO	Rarefied_PSSB
<i>Apatania</i> sp.		X	X	X
<i>Cinygma</i> sp.			X	
<i>Cinygmula</i> sp.				X
Elmidae	X	X		
<i>Hesperoperla pacifica</i>	X	X		X
<i>Heterlimnius corpulentus</i>	X	X		
<i>Ironodes</i> sp.				X
<i>Lara</i> sp.	X	X		
* <i>Margaritifera falcata</i>	X	X	X	
<i>Narpus concolor</i>	X	X		
<i>Neophylax splendens</i>				X
<i>Optioservus</i> sp.	X	X		
<i>Rhithrogena</i> sp.				X
<i>Sweltsa</i> sp.		X		
<i>Tipula</i> sp.		X		
<i>Zaitzevia</i> sp.		X		

Note: *Indicates this taxon is excluded from calculation of the B-IBI score using individual biometrics.

As Figure 2 shows, new taxa are not identified in appreciable numbers beyond the 500-count, therefore their influence on the richness biometrics is negligible. Since sampling is at a limited number of locations within a reach and assuming the widest variety of riffle conditions are being sampled, and the composited sample is being sub-sampled for development of a taxa list and estimated densities, retaining as much biological information as possible will maximize the potential to understand the relationship between taxa richness and number of organisms identified (Figure 2Error! Reference source not found.). Rare taxa representing the Long-Lived and Intolerant species are not abundant in samples so counting as many organisms as possible will maximize our representation of these taxa while adhering to sub-sampling rules.

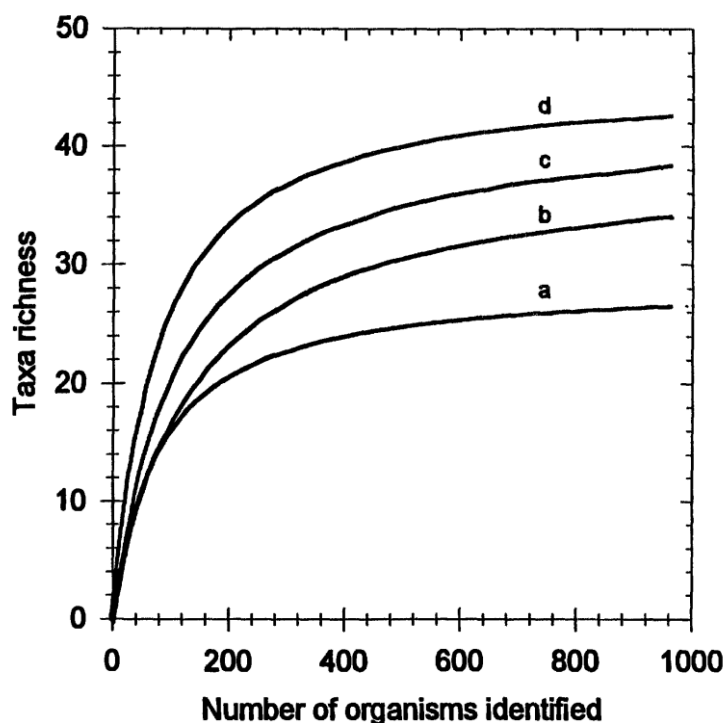


Figure 2 TAXA RICHNESS VERSUS SUB-SAMPLE COUNT USING DATA FROM THE BUG LAB; CURVES A THROUGH D REPRESENT DIFFERING SAMPLE COLLECTION AREAS (VINSON AND HAWKINS 1996), A=0.05-0.1M², B=0.1-0.5M², C=0.5-1.0M², OR D=>1.0M².

The use of rarefaction when full count samples contain between 500 and 600 organisms may not be necessary when comparing with other samples using the same area of collection and laboratory sampling procedures (Vinson and Hawkins 1996). Unintended results of rarefaction at degraded sites may result in the elimination of taxa and/or change densities in a way that results in higher B-IBI scores (McCabe and Gotelli 2000). This may not always be the case in western Washington streams and requires further investigation.

The PSSB database uses a complex method for applying rarefaction to whole sub-samples when re-expressing the taxa list based on a fixed 500-count. The rarefaction method standardizing the sub-sample to 500-count cannot be duplicated at this time outside of the PSSB application. Currently, an evaluation of the PSSB tool for calculating B-IBI using a rarefaction routine is under review with a goal to make it transparent and tested for repeatability. The B-IBI calculator adopted by PSSB could then be provided as a separate tool (R code) for use independently by monitoring organizations.

Based on previous research and effect on taxa lists, the SOW monitoring program will continue to generate biometric scores based on full count samples (≥ 500 -organisms) and biometric categorizations based on structural/functional/behavioral characteristics established by Rhithron Associates, Inc (our taxonomic consultant). Using full count data enables interpretation of site condition based on all species present and preserves as much biological information as possible which may be advantageous when interpreting B-IBI scores that are at thresholds between condition categories.

Autecological designations and species names recognized by our primary source of taxonomic information (Rhithron Associates, Inc.) will be the basis for how benthic macroinvertebrate data is prepared prior to calculation of B-IBI scores. When future changes to taxonomic designations occur, and comparison to prior or historical data is desired, a re-analysis of that data using current taxonomic designations will be required in order to analyze for trends in biological condition.

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APPENDIX 1. R CODE THAT CAN BE USED TO GENERATE A SPECIES LIST AND DENSITIES BASED ON RAREFACTION OF A 500-COUNT SUB-SAMPLE (THIS IS A SIMPLE RAREFACTION ROUTINE THAT DOES NOT INCLUDE SOME OF THE SPECIAL FUNCTIONS CONTAINED IN THE PSSB DATABASE).

```
library(BioMonTools)
library(knitr)

# set directory, upload data frame
wd <- file.path("C:/Users/rober/Documents/Rarefaction")
myFile <- "SNOCO2018_Orig1.csv"
df_biodata <- read.csv(file.path(wd, myFile))

# set number of organisms and seed (if you want reproducible results; otherwise each run is random and will be different)
mySize <- 500
Seed_US <- 17760704
bugs_mysize <- rarify(inbug=df_biodata, sample.ID="SampleID", abund="N_TAXA", subsiz=mySize, mySeed=Seed_US)

# Save output
myFile.Out <- paste0("SNOCO2018_Rarify1_500", mySize, ".csv")
write.csv(bugs_mysize, file=myFile.Out, row.names=FALSE)

#read in the rarified csv
myFile <- "SNOCO2018_Rarify1_500.csv"
bugs_mysize <- read.csv(file.path(wd, myFile))

# create pre- and post- subsample comparison data frame
df_compare <- merge(df_biodata, bugs_mysize, by=c("SampleID", "TaxaID"), suffixes = c("_Orig", "_500"))

# compare totals
tbl_totals <- aggregate(cbind(N_TAXA_Orig, N_TAXA_500) ~ SampleID, df_compare, sum)
kable(head(tbl_totals), caption = "Comparison, sample totals")

# Compare pre- and post- subsample taxa counts
df_compare <- df_compare[,c("SampleID", "TaxaID", "N_TAXA_Orig", "N_TAXA_500")]
kable(head(df_compare), caption = "Comparison, by Sample")

# Save output
```

```
write.csv(df_compare, paste("Compare",mySize,"csv",sep="."))
```

APPENDIX 2. 2018 TAXONOMIC LIST FOR SCOTT CREEK (SCTT), SNOHOMISH BASIN (*ELIMINATED FOLLOWING RAREFACTION; PUPA & ADULTS EXCLUDED FROM ANALYSIS).

Common Name	Species – Scientific Name	Life Stage
Non-Insect Taxa	<i>Polycelis</i>	Larval
Non-Insect Taxa	Nemata	Larval
Non-Insect Taxa	<i>Margaritifera falcata</i>	Larval
Non-Insect Taxa	Acari	Larval
Non-Insect Taxa	Oligochaeta	Larval
Odonata (Dragonflies)	<i>Cordulegaster dorsalis</i>	Larval
Ephemeroptera (Mayflies)	Baetis tricaudatus complex	Larval
Ephemeroptera (Mayflies)	<i>Dipheter hageni</i>	Larval
Ephemeroptera (Mayflies)	<i>Ephemerella tibialis</i>	Larval
Ephemeroptera (Mayflies)	<i>Cinygma</i>	Larval
Ephemeroptera (Mayflies)	<i>Cinygmula</i>	Larval
Ephemeroptera (Mayflies)	Heptageniidae - early instar or damaged	Larval
Ephemeroptera (Mayflies)	<i>Ironodes</i>	Larval
Ephemeroptera (Mayflies)	<i>Rhithrogena</i>	Larval
Ephemeroptera (Mayflies)	Leptophlebiidae - early instar or damaged	Larval
Plecoptera (Stoneflies)	<i>Sweltsa</i>	Larval
Plecoptera (Stoneflies)	<i>Malenka</i>	Larval
Plecoptera (Stoneflies)	<i>Zapada cinctipes</i>	Larval
Plecoptera (Stoneflies)	Zapada Oregonensis Gr.	Larval
Plecoptera (Stoneflies)	<i>Hesperoperla pacifica</i>	Larval
Plecoptera (Stoneflies)	Perlodidae - early instar	Larval
Plecoptera (Stoneflies)	<i>Skwala</i>	Larval
Trichoptera (Caddisflies)	*Trichoptera - damaged pupa	Pupa
Trichoptera (Caddisflies)	<i>Apatania</i>	Larval
Trichoptera (Caddisflies)	<i>Micrasema</i>	Larval
Trichoptera (Caddisflies)	<i>Glossosoma</i>	Larval
Trichoptera (Caddisflies)	<i>Hydropsyche</i>	Larval
Trichoptera (Caddisflies)	*Hydropsychidae - early instar or pupa	Pupa
Trichoptera (Caddisflies)	<i>Lepidostoma</i>	Larval
Trichoptera (Caddisflies)	<i>Dicosmoecus gilvipes</i>	Larval
Trichoptera (Caddisflies)	<i>Wormaldia</i>	Larval
Trichoptera (Caddisflies)	* <i>Rhyacophila</i> - early instar or pupa or damaged	Pupa
Trichoptera (Caddisflies)	<i>Rhyacophila Betteni</i> Gr.	Larval
Trichoptera (Caddisflies)	<i>Rhyacophila blarina</i>	Larval

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Common Name	Species – Scientific Name	Life Stage
Trichoptera (Caddisflies)	Rhyacophila Brunnea/Vemna Gr.	Larval
Trichoptera (Caddisflies)	<i>Rhyacophila narvae</i>	Larval
Trichoptera (Caddisflies)	<i>Neophylax splendens</i>	Larval
Coleoptera (Beetles)	Elmidae - early instar or damaged	Larval
Coleoptera (Beetles)	<i>Heterolimnius corpulentus</i>	Larval
Coleoptera (Beetles)	<i>Lara</i>	Larval
Coleoptera (Beetles)	<i>Narpus concolor</i>	Larval
Coleoptera (Beetles)	<i>Optioservus</i>	Larval
Coleoptera (Beetles)	<i>Zaitzevia</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Ceratopogoninae	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Forcipomyiinae	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Dixa</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Psychodidae - early instar	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Simulium</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Dicranota</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Tipula</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Polypedilum</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Micropsectra</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Rheotanytarsus</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Stempellinella</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Brillia</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Corynoneura</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Cricotopus / Orthocladius</i> - early instar	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Eukiefferiella Claripennis Gr.	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Eukiefferiella Coerulescens Gr.	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Eukiefferiella tirolensis</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Parametriocnemus</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Synorthocladius</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Tvetenia Bavarica Gr.	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Pentaneura</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	*Thienemannimyia Gr. - early instar	Larval

APPENDIX 3. 2018 TAXA LIST FOR NORTH CREEK AT CLEARWATER SCHOOL (NCCW), LAKE WASHINGTON BASIN BASIN
(*ELIMINATED FOLLOWING RAREFACTION; PUPA & ADULTS EXCLUDED FROM ANALYSIS).

Common Name	Species – Scientific Name	Life Stage
Non-insect Taxa	Acari	Unknown
Amphipoda	<i>Crangonyx</i>	Unknown
Ephemeroptera	<i>Baetis tricaudatus</i> complex	Larval
Ephemeroptera	<i>Dipheter hageni</i>	Larval
Ephemeroptera	<i>Ephemerella</i>	Larval
Ephemeroptera	<i>Serratella tibialis</i>	Larval
Plecoptera	<i>Hesperoperla pacifica</i>	Larval
Plecoptera	<i>Skwala</i>	Larval
Plecoptera	<i>Suwalliini</i>	Larval
Plecoptera	<i>Sweltsa</i>	Larval
Plecoptera	<i>Zapada cinctipes</i>	Larval
Trichoptera	<i>Glossosoma</i>	Larval
Trichoptera	*Glossosomatidae	Pupa
Trichoptera	<i>Hydropsyche</i>	Larval
Trichoptera	<i>Hydroptila</i>	Larval
Trichoptera	*Hydroptilidae	Pupa
Trichoptera	<i>Micrasema</i>	Larval
Trichoptera	* <i>Rhyacophila</i>	Pupa
Trichoptera	<i>Rhyacophila</i> Betteni Group	Larval
Trichoptera	<i>Rhyacophila blarina</i>	Larval
Trichoptera	<i>Rhyacophila</i> Brunnea/Vemna Group	Larval
Coleoptera	* <i>Cleptelmis addenda</i>	Adult
Coleoptera	<i>Heterlimnius corpulentus</i>	Larval
Coleoptera	<i>Narpus concolor</i>	Larval
Coleoptera	* <i>Optioservus</i>	Adult
Diptera	<i>Antocha monticola</i>	Larval
Diptera	<i>Chelifera</i>	Larval
Diptera	Chironomini	Larval
Diptera	<i>Dicranota</i>	Larval
Diptera	*Empididae	Pupa
Diptera	<i>Eukiefferiella</i> Brehmi Group	Larval
Diptera	<i>Pagastia</i>	Larval
Diptera	<i>Polypedilum</i>	Larval
Diptera	<i>Potthastia</i> Gaedii Group	Larval
Diptera	<i>Rheotanytarsus</i>	Larval
Diptera	<i>Simulium</i>	Larval
Diptera	<i>Thienemannimyia</i> complex	Larval
Diptera	<i>Tvetenia</i> Bavarica Group	Larval

APPENDIX 4. TAXONOMIC LIST FOR FRENCH CREEK (FCSP), SNOHOMISH BASIN BASIN (*ELIMINATED FOLLOWING RAREFACTION; PUPA & ADULTS EXCLUDED FROM ANALYSIS).

Common Name	Species – Scientific Name	Life Stage
Veneroida (Pea Clams/Fingernail Clams)	Sphaeriidae	Larval
Non-Insect Taxa	Nemata	Larval
Non-Insect Taxa	Acari	Larval
Non-Insect Taxa	Oligochaeta	Larval
Ephemeroptera (Mayflies)	Baetidae - early instar or damaged	Larval
Ephemeroptera (Mayflies)	Baetis tricaudatus complex	Larval
Ephemeroptera (Mayflies)	<i>Diphetor hageni</i>	Larval
Ephemeroptera (Mayflies)	<i>Drunella doddsii</i>	Larval
Ephemeroptera (Mayflies)	<i>Cinygma</i>	Larval
Ephemeroptera (Mayflies)	<i>Cinygmula</i>	Larval
Ephemeroptera (Mayflies)	<i>Ironodes</i>	Larval
Ephemeroptera (Mayflies)	<i>Rhithrogena</i>	Larval
Ephemeroptera (Mayflies)	Leptophlebiidae - early instar or damaged	Larval
Plecoptera (Stoneflies)	Chloroperlidae - early instar	Larval
Plecoptera (Stoneflies)	<i>Sweltsa</i>	Larval
Plecoptera (Stoneflies)	*Leuctridae - early instar or damaged	Larval
Plecoptera (Stoneflies)	<i>Malenka</i>	Larval
Plecoptera (Stoneflies)	<i>Soyedina</i>	Larval
Plecoptera (Stoneflies)	<i>Zapada cinctipes</i>	Larval
Plecoptera (Stoneflies)	<i>Zapada Oregonensis</i> Gr.	Larval
Plecoptera (Stoneflies)	<i>Calineuria californica</i>	Larval
Plecoptera (Stoneflies)	<i>Hesperoperla pacifica</i>	Larval
Plecoptera (Stoneflies)	*Perlidae - early instar	Larval
Plecoptera (Stoneflies)	<i>Kogotus</i>	Larval
Plecoptera (Stoneflies)	Perlodidae - early instar	Larval
Plecoptera (Stoneflies)	<i>Skwala</i>	Larval
Plecoptera (Stoneflies)	<i>Pteronarcys</i> - early instar	Larval
Plecoptera (Stoneflies)	<i>Pteronarcys princeps</i>	Larval
Trichoptera (Caddisflies)	<i>Micrasema</i>	Larval
Trichoptera (Caddisflies)	<i>Glossosoma</i>	Larval
Trichoptera (Caddisflies)	*Glossosomatidae - pupa	Pupa
Trichoptera (Caddisflies)	<i>Hydropsyche</i>	Larval
Trichoptera (Caddisflies)	*Hydropsychidae - early instar or pupa	Pupa
Trichoptera (Caddisflies)	<i>Lepidostoma</i>	Larval
Trichoptera (Caddisflies)	Limnephilidae - early instar or pupa	Pupa
Trichoptera (Caddisflies)	<i>Wormaldia</i>	Larval
Trichoptera (Caddisflies)	* <i>Rhyacophila</i> - early instar or pupa or damaged	Pupa
Trichoptera (Caddisflies)	<i>Rhyacophila Betteni</i> Gr.	Larval
Trichoptera (Caddisflies)	<i>Rhyacophila blarina</i>	Larval
Trichoptera (Caddisflies)	<i>Rhyacophila narvae</i>	Larval
Trichoptera (Caddisflies)	<i>Neophylax splendens</i>	Larval
Coleoptera (Beetles)	<i>Cleptelmis addenda</i>	Larval
Coleoptera (Beetles)	Elmidae - early instar or damaged	Larval

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Common Name	Species – Scientific Name	Life Stage
Coleoptera (Beetles)	<i>Heterlimnius corpulentus</i>	Larval
Coleoptera (Beetles)	<i>Lara</i>	Larval
Coleoptera (Beetles)	<i>Narpus concolor</i>	Larval
Coleoptera (Beetles)	<i>Optioservus</i>	Larval
Coleoptera (Beetles)	<i>Zaitzevia</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Dixa</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Simulium</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Tipula</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Microtendipes</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Polypedilum</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Micropsectra</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Rheotanytarsus</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Tanytarsus</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Brillia</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Corynoneura</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Cricotopus (Cricotopus)</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>*Eukiefferiella Claripennis Gr.</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>*Orthoclaadiinae - early instar or damaged pupa</i>	Pupa
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Parametriocnemus</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Rheocricotopus</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Tvetenia - pupa</i>	Pupa
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Tvetenia Bavarica Gr.</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Thienemannimyia Gr. - early instar</i>	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	<i>Zavreliomyia</i>	Larval

APPENDIX 5. 2018 TAXONOMIC LIST FOR EAST FORK WOODS CREEK (WDOP), SNOHOMISH BASIN BASIN (*ELIMINATED FOLLOWING RAREFACTION; PUPA & ADULTS EXCLUDED FROM ANALYSIS).

Common Name	Species – Scientific Name	Life Stage
Veneroida (Pea Clams/Fingernail Clams)	Sphaeriidae	Larval
Non-Insect Taxa	Acari	Larval
Ephemeroptera (Mayflies)	Anafroptilum	Larval
Ephemeroptera (Mayflies)	Baetidae - early instar or damaged	Larval
Ephemeroptera (Mayflies)	Baetis tricaudatus complex	Larval
Ephemeroptera (Mayflies)	Dipheter hageni	Larval
Ephemeroptera (Mayflies)	Attenella delantala	Larval
Ephemeroptera (Mayflies)	Serratella micheneri	Larval
Ephemeroptera (Mayflies)	Timpanoga hecuba	Larval
Ephemeroptera (Mayflies)	Cinygmula	Larval
Ephemeroptera (Mayflies)	Heptageniidae - early instar or damaged	Larval
Ephemeroptera (Mayflies)	Rhithrogena	Larval
Ephemeroptera (Mayflies)	Paraleptophlebia bicornuta	Larval
Plecoptera (Stoneflies)	Capniidae - early instar	Larval
Plecoptera (Stoneflies)	Paraperlinae - early instar	Larval
Plecoptera (Stoneflies)	Suwalliini - early instar	Larval
Plecoptera (Stoneflies)	Sweltsa	Larval
Plecoptera (Stoneflies)	Zapada cinctipes	Larval
Plecoptera (Stoneflies)	Isoperla	Larval
Plecoptera (Stoneflies)	Perlodidae - early instar	Larval
Plecoptera (Stoneflies)	Skwala	Larval
Plecoptera (Stoneflies)	Pteronarcys - early instar	Larval
Trichoptera (Caddisflies)	*Trichoptera - damaged pupa	Pupa
Trichoptera (Caddisflies)	Apatania	Larval
Trichoptera (Caddisflies)	Amiocentrus aspilus	Larval
Trichoptera (Caddisflies)	Brachycentridae - early instar	Larval
Trichoptera (Caddisflies)	Agapetus	Larval
Trichoptera (Caddisflies)	Glossosoma	Larval
Trichoptera (Caddisflies)	Hydropsyche	Larval
Trichoptera (Caddisflies)	*Hydropsychidae - early instar or pupa	Pupa
Trichoptera (Caddisflies)	Parapsyche	Larval
Trichoptera (Caddisflies)	Hydroptila	Larval
Trichoptera (Caddisflies)	Wormaldia	Larval
Trichoptera (Caddisflies)	Rhyacophila blarina	Larval
Trichoptera (Caddisflies)	Rhyacophila malkini	Larval
Coleoptera (Beetles)	Dytiscidae - larva	Larval
Coleoptera (Beetles)	Oreodytes	Larval
Coleoptera (Beetles)	Cleptelmis addenda	Larval
Coleoptera (Beetles)	Elmidae - early instar or damaged	Larval
Coleoptera (Beetles)	Heterlimnius corpulentus	Larval
Coleoptera (Beetles)	Lara	Larval
Coleoptera (Beetles)	Narpus concolor	Larval
Coleoptera (Beetles)	Optioservus	Larval

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Common Name	Species – Scientific Name	Life Stage
Veneroida (Pea Clams/Fingernail Clams)	Sphaeriidae	Larval
Coleoptera (Beetles)	Zaitzevia	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Dixa	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Hemerodromia	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Neoplasia	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Simulium	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Antocha monticola	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Dicranota	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Microtendipes	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Phaenopsectra	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Polypedilum	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Cladotanytarsus	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Micropsectra	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Paratanytarsus	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Rheotanytarsus	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Stempellinella	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	*Tanytarsini - early instar or damaged pupa	Pupa
Diptera (Mosquitoes, Biting Midges, Blackflies)	Tanytarsus	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Pagastia	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Potthastia Gaedii Gr.	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Brillia	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Corynoneura	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	*Cricotopus - damaged or pupa	Pupa
Diptera (Mosquitoes, Biting Midges, Blackflies)	Cricotopus / Orthocladius - early instar	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Eukiefferiella - early instar	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Eukiefferiella Brehmi Gr.	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Parakiefferiella	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Parametriocnemus	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Psectrocladius	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Rheocricotopus	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Synorthocladius	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Thienemanniella	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	*Tvetenia - pupa	Pupa
Diptera (Mosquitoes, Biting Midges, Blackflies)	Tvetenia Bavarica Gr.	Larval
Diptera (Mosquitoes, Biting Midges, Blackflies)	Thienemannimyia Gr. - early instar	Larval

APPENDIX 6. 2018 TAXONOMIC LIST FOR TRAFTON CREEK (TRFT), STILLAGUAMISH BASIN BASIN (*ELIMINATED FOLLOWING RAREFACTION; PUPA & ADULTS EXCLUDED FROM ANALYSIS).

Common Name	Species – Scientific Name	Life Stage
Water Mites	Acari	Unknown
Roundworms	Nemata	Unknown
Basommatophora (Snails)	Menetus	Unknown
Coleoptera (Beetles)	*Oreodytes	Adult
Coleoptera (Beetles)	Cleptelmis addenda	Larva
Coleoptera (Beetles)	Lara	Larva
Coleoptera (Beetles)	Narpus concolor	Larva
Coleoptera (Beetles)	*Optioservus	Adult
Coleoptera (Beetles)	Optioservus	Larva
Coleoptera (Beetles)	*Zaitzevia	Adult
Coleoptera (Beetles)	Zaitzevia	Larva
Coleoptera (Beetles)	*Hydraena	Adult
Diptera (Mosquitoes, Biting Midges, Blackflies)	Ceratopogoninae	Larva
Diptera (Mosquitoes, Biting Midges, Blackflies)	Natarsia	Larva
Diptera (Mosquitoes, Biting Midges, Blackflies)	Odontomesa	Larva
Diptera (Mosquitoes, Biting Midges, Blackflies)	Polypedilum	Larva
Diptera (Mosquitoes, Biting Midges, Blackflies)	Thienemannimyia Gr.	Larva
Diptera (Mosquitoes, Biting Midges, Blackflies)	Tvetenia Bavarica Gr.	Larva
Diptera (Mosquitoes, Biting Midges, Blackflies)	Dixa	Larva
Diptera (Mosquitoes, Biting Midges, Blackflies)	*Empididae	Pupa
Diptera (Mosquitoes, Biting Midges, Blackflies)	Trichoclinocera	Larva
Diptera (Mosquitoes, Biting Midges, Blackflies)	Ptychoptera	Larva
Diptera (Mosquitoes, Biting Midges, Blackflies)	Simulium	Larva
Diptera (Mosquitoes, Biting Midges, Blackflies)	Dicranota	Larva
Ephemeroptera (Mayflies)	Baetis tricaudatus complex	Larva
Ephemeroptera (Mayflies)	Dipheter hageni	Larva
Ephemeroptera (Mayflies)	Ephemerella	Larva
Plecoptera (Stoneflies)	Malenka	Larva
Plecoptera (Stoneflies)	Hesperoperla pacifica	Larva
Plecoptera (Stoneflies)	Skwala	Larva
Trichoptera (Caddisflies)	Onocosmoecus unicolor	Larva
Trichoptera (Caddisflies)	Rhyacophila blarina	Larva
Trichoptera (Caddisflies)	Rhyacophila Brunnea/Vemna Gr.	Larva
Trichoptera (Caddisflies)	Neophylax splendens	Larva
Veneroida (Pea Clams/Fingernail Clams)	Sphaeriidae	Unknown